Int J Clin Exp Med 2015;8(6):9205-9217
www.ijcem.com /ISSN:1940-5901/IJCEM0008403

Original Article

Radix Bupleuri ameliorates depression by increasing nerve growth factor and brain-derived neurotrophic factor

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Received March 24, 2015; Accepted June 3, 2015; Epub June 15, 2015; Published June 30, 2015

Abstract: Background: Chinese herb *Radix Bupleuri* has been regarded effective to improve treatment of depression, but the molecular mechanism remains unknown. Low levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) increase the likelihood of developing the depression. Therefore, we want to know whether *Radix Bupleuri* affects the levels of these factors. Methods: A total 160 hemodialysis patients were diagnosed with depression and randomly assigned to two groups: *Radix Bupleuri* group (received 1 g root power of *Radix Bupleuri* in a capsule daily *Radix Bupleuri*) and control group (receive placebo). Results: After three-month follow-up, the patients who received *Radix Bupleuri* had greater improvement in depression symptoms, anxiety symptoms and general functioning via controls after three-month follow-up ($P < 0.05$). Serum NGF levels were significantly higher in subjects accepted *Radix Bupleuri*s (178.64 ± 52.18 pg/mL) when compared to a control (103.54 ± 31.23 pg/ml) ($P < 0.01$). Similarly, serum BDNF levels were significantly higher in subjects accepted *Radix Bupleuri*s (1635.26 ± 121.66 pg/ml) when compared to a control (516.38 ± 44.89 pg/ml) ($P < 0.01$). The serum levels of NGF and BDNF were negatively related with Montgomery-Asberg Depression Rating Scale (MADRS) and positively related with scores of RAND-36 item Health Survey (RAND-36) ($P < 0.01$). Conclusion: Thus, *Radix Bupleuri* ameliorates the patients with depression by increasing serum levels of NGF and BDNF. *Radix Bupleuri* should be developed a new drug for the therapy of depression.

Keywords: Brain-derived neurotrophic factor, depression, montgomery-asberg depression rating scale, nerve growth factor, *Radix Bupleuri*

Introduction

Depression is a global burden disease, which greatly affects the quality of life and causes the loss of general functioning [1]. Depression was the third leading cause of the global burden of diseases in 2004 and the disorder will be the first leading cause of the global burden disease by 2030 [2]. The causes of depression are complex and related mechanisms are widely unknown. For example, hypertension and diabetes are the primary etiologies accounting for renal failure [3]. The end-stage renal disease is often handled with hemodialysis. Depression has been declared to be associated with hemodialysis patients [4].

Medicine therapy is the most common way for the treatment of depression [5]. However, there are still some problems for the therapy of depression using medicine [6]. Most medicine has significant side effects, which limit its utilization. For example, aripiprazole can significantly and rapidly improve the core depressive symptoms [7]. However, the medicine can cause many normal side effects such as severe hyponatremia [8], dizziness [9] and drowsiness [10] so on. Selective serotonin reuptake inhibitors (SSRIs) are the currently most used drugs to treat depression because they generally have fewer adverse effects than most other antidepressants. However, the side effects of SSRIs have also been widely reported. For example, erectile dysfunction is one of common side effects reported by men treated with SSRIs [11]. Another example, some people who take SSRIs report that they have experienced 'blunted' emotions. Emotional side-effect of SSRIs is

a robust phenomenon, prominent in patients' thoughts about their medication, making an incontrovertible impact on their functions and acting as an important role in their decision-making about antidepressant adherence [12]. Thus, it is necessary to find a more effective way for the therapy of depression.

Chinese herbs can ameliorate depression and delay the deterioration of cognitive functions in elderly persons with dementia with a few side effects [13, 14]. It has been proved that the effectiveness of Chinese herb *Radix Bupleuri* as a medicine intervention for the therapy of functional dyspepsia accompanied with depression [15], but the molecular mechanism is still unknown. Low levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) increase the likelihood of developing the depression [16-19]. However, the clinical relevance of *Radix Bupleuri* and the levels of NGF and BDNF have never been reported. The depression of hemodialysis patients is widely reported and difficult to be treated [20]. To address the problem, *Radix Bupleuri* therapy can be developed for the therapy of depression. Meanwhile, we investigated whether there were some relationships between *Radix Bupleuri* and the levels of NGF or BDNF.

Methods

Participants

The procedures of the study were proved by Research Ethics Committee and Research and Publication Communication Committee from First Affiliated Hospital of Dalian Medical University (registration number is DLM201-3090608). Two hundred and eighty hemodialysis patients from Chinese Han were enrolled at blood purification center of the First Affiliated Hospital of Dalian Medical University (Dalian, China) from May 2013 to September 2013. All patients should sign informed consent before they were recruited.

Inclusion criteria

All participants (30-55 years) was evaluated using the Kimberley Indigenous Cognitive Assessment of Depression (KICA-dep) [21] according to the World Health Organization's International classification of Diseases and the American Psychiatric Association's, the Fourth Edition-Text Revision (DSM-IV-TR) [22]. There were 11 items for KICA-dep, each of which was rated from 'never' to 'sometimes', 'a lot' and 'all the time' according to a frequency scale. Anxiety was considered because anxiety tends to precede onset of comorbid depression and to promote depressive episodes of the frequent comorbidity of depression and anxiety [23].

There are numerous factors for causing depression. In order to reduce the interference of these factors on the results, age [24], gender [25], career [26], renal failure [27], diabetes [28] and the months of hemodialysis [29] was compared between the control group and *Radix Bupleuri* group. Furthermore, to exclude the effects of other factors, all subjects were recruited from the same city, Dalian (in the northeast of China), and live in the families with similar educational and economic background.

Exclusion criteria

The following patients were not included if they had: (1) a history of repeated suicidal behavior; (2) acute and severe substance abuse; difficulty in verbal conversation because of severe depression; (3) a family history of depression; (4) brain injury or other brain diseases.

Study design

The roots of Radix Bupleuri were from Jinbaoan Trade Co., Ltd. (Zhunan Township, Taiwan). After the scanning of patients with depression, 160 patients were eligible to enter the three-month follow-up phase and randomly divided into *Radix Bupleuri* group (would receive 1 g of the root powder of Radix Bupleuri in a capsule daily) and control group (would receive placebo). Here the study was performed with medical controlled trial with two parallel experiments (**Figure 1**). The left part showed the depression patients only receiving placebo therapy. The right part of the figure showed the patients receiving the *Radix Bupleuri* from recruitment to three-month follow-up.

The effects of *Radix Bupleuri* were compared to those of control group. There were 80 subjects to be included in each group. Of these, eighteen patients from the control group and 5 patients from *Radix Bupleuri* group dropped out during the therapy because of no motivation and other unknown factors (**Figure 1**).

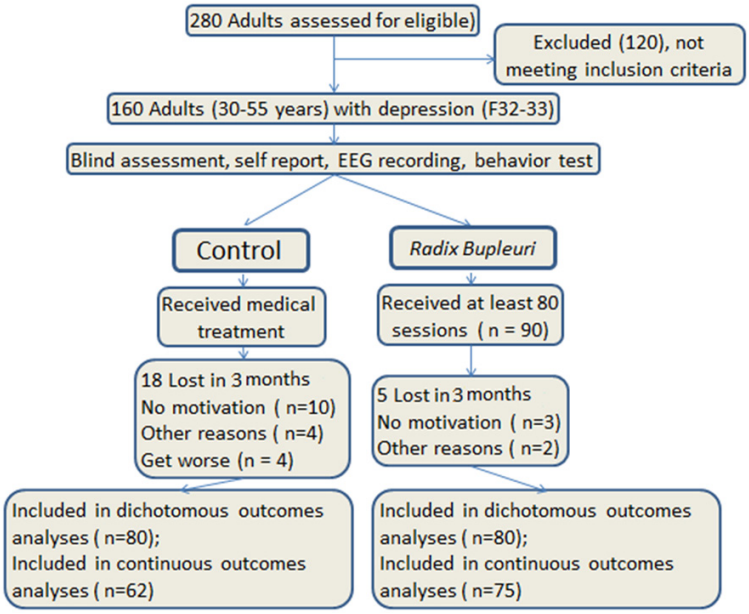

Figure 1. Flowchart of study participants with three-month follow-up.

Among these patients, four patients dropped out from the control group because their depression got worse during the three-month follow-up.

Outcome

The depression degrees were examined based on the Montgomery and Asberg Depression Rating Scale (MADRS) [30]. There are ten items for MADRS, and the score ranges from 0 to 60. High joint reliability (from 0.75 to 0.96) and the sensitivity to change were shown in these studies. Predictive validity for depression disease was demonstrated, and the depression was defined as severe, moderate, and mild according to cut-off scores [31].

Anxiety is often accompanied with depression [29], and here the anxiety was examined based on the Hospital Anxiety and Depression Scale (HADS) [32]. There are seven items in the questionnaire of anxiety subscale (HADS-A). The scores of HADS-A varies from 0 to 21, and higher scores stand for more anxiety. General functioning was investigated using a global assessment of functioning (GAF) according to a previous report [33]. Life quality was assessed based on the RAND-36 according to a previous report [34], which analyzes well-being and functioning from eight dimensions. Psychopathological dysfunction in emotion is often described using alexithymia, which is also related with depression. Here alexithymia was investigated based on the Toronto Alexithymia Scale-20 (TAS-20) [35].

ELISA analysis of serum NGF and BDNF

To determine serum levels of NGF and BDNF, ten milliliters of blood were withdrawn (8:00 to 11:00 a.m.) by venipuncture into a free-anticoagulant vacuum tube before and after three-month follow-up. The blood was immediately centrifuged at 4000 g for 15 min, and serum was frozen at 80°C. Serum levels of NGF and BDNF were measured using an NGF ELISA kit (Shanghai Wuhao Trade Co. Ltd., Shanghai, China) and BDNF ELISA kit (R&D Systems China, Shanghai, China). The intra-assay coefficient of variation (C.V.) was lower than 5 percent and the inter assay C.V. was lower than 10 percent. Additionally, the assay sensitivity was one pg/ml and the detection limitation was 4.0 pg/ml. Serum levels of NGF and BDNF below the detection limit were considered to be 0. All values were counted as ± pg/ml.

Data analyses

All results emerged as continuous or dichotomous outcomes. For dichotomous outcomes, it means that the outcome is achieved when some information is missing. For continuous outcomes, it means that all the data are retained from all participants whose information is available. All the outcomes were analyzed according to the method of intention-to-treat [36]. Fisher's exact test was done for odds ratios with 95% confidence intervals of dichotomous outcomes. Welch's t-test was carried out with 95% confidence intervals for the changes of continuous outcomes. The *t*-test is conducted to assess whether the expected values of a quantitative variable within both groups differ from each other. These data were analyzed using SPSS 10.0 package. Spearman's rank correlation coefficient was used to identify the strength of correlation between the degrees of

Table 1. Baseline characteristics of patients

	Radix Bupleuri (n = 80)	Control (n = 80)	Mean difference (95% CI)	Odds ratio (95% CI)	*P* value
Age: years, mean (s.d.)	41.9 (11.8)	42.5 (12.5)	0.58 (-5.60 to 4.89)		0.90
Male, n (%)	21 (25.1)	22 (25.2)		1.45 (0.28 to 5.27)	0.84
Smoker/Non-smoker	24/80	25/80		1.29 (0.31 to 2.98)	0.47
Drinker/Non-drinker	23/80	21/80		1.63 (0.26 to 3.88)	0.66
Renal failure	53 (66)	55 (69)		1.25 (0.37 to 3.29)	0.59
Diabetes	27 (34)	25 (31)		1.34 (0.54 to 5.17)	0.48
Education levels (> 9 years), n (%)	11 (14)	13 (16)		1.39 (0.43 to 4.86)	0.53
Spouse, n (%)	62 (78)	58 (73)		1.31 (0.25 to 3.77)	0.64
Career (working overtime), n (%)	51 (64)	54 (68)		1.42 (0.41 to 5.24)	0.52
Months of hemodialysis , n (s.d)	26 (13)	29 (15)		1.21 (0.50 to 5.57)	0.38
Anxiety (cut-off score 8 in Hospital Anxiety and Depression Scale), n (%)	68 (85)	71 (89)		1.51 (1.43 to 1.59)	0.51
Current medication, n (%)					
Antidepressant medication	41 (51)	37 (46)		1.25 (0.35 to 3.44)	0.32
Selective serotonin reuptake inhibitors	27 (34)	32 (40)		0.83 (0.51 to 2.39)	0.54
Serotonin and noradrenaline reuptake inhibitors	12 (15)	11 (14)		1.42 (0.53 to 3.85)	0.85
Psychiatric test scores, mean (s.d.)					
Montgomery-Asberg Depression Rating Scale	23.9 (7.8)	24.6 (7.4)	1.59 (75.1 to 1.87)		0.33
Anxiety score (Hospital Anxiety and Depression Scale)	12.6 (4.5)	11.3 (4.1)	1.02 (74.6 to 0.81)		0.27
Global Assessment of Functioning score	59.4 (6.8)	58.1 (8.3)	1.12 (73.4 to 3.2)		0.65
Toronto Alexithymia Scale-20	54.6 (13.8)	53.7 (14.3)	1.51 (77.2 to 5.4)		0.71
RAND-36 score	52.3 (16.1)	53.6 (15.9)	2.24 (75.6 to 7.8)		0.60

Table 2. The reduction of primary and secondary outcomes in the *Radix Bupleuri* group[a]

Outcome	3-month follow-up		
	n/N (%)	Odds ratio (95% CI)	P
Leaving the study early			
Control group	19/80 (24)	0.46 (0.16-1.94)	0.45
Radix Bupleuri group	7/80 (9)		
Response[b]			
Control group	21/80 (26)	3.22 (1.24-8.19)	0.02
Radix Bupleuri group	45/80 (56)		

a. Missing information was treated as no response (intention-to-treat analysis). b. Response was defined as a 50% or greater reduction in Montgomery-Asberg Depression Rating Scale symptom scores.

depression (Montgomery-Asberg Depression Rating Scale or RAND-36) and the levels of NGF or BDNF. Software can be found at http://www.wessa.net/rwasp_spearman.wasp.

Results

Baseline characteristics of participants

Two hundred and eighty subjects were evaluated for the depression in hemodialysis patients and 80 patients were excluded after scanning using inclusion and exclusion criteria. Thus, 160 subjects were randomly divided into *Radix Bupleuri* group and control group evenly. Based on the comparison of the control group and *Radix Bupleuri* group, there were no significant differences for the compound factors affecting depression between two groups (**Table 1**).

Similarly, the values were also similar between control group and *Radix Bupleuri* group for psychiatric test and the prevalence of KICA-dep items among hemodialysis patients with ICD-10 and DSM-IV-TR diagnosis of depression ($P > 0.05$) (**Tables 1** and **4**). All these results, the baseline characters of patients were similar between two groups, which would not interfere with the results of the *Radix Bupleuri*.

The decrease in outcomes diagnosed in the Radix Bupleuri group for dichotomous outcomes

In the treatment of depression, outcomes can be evaluated based on dichotomous outcomes or continuous ones. However, the consequences of the two outcomes cannot be used interchangeably due to differences in heterogeneity and subgroup analyses [37]. Thus, the dichotomous outcomes were analyzed separately. There were 160 cases from control groups and *Radix Bupleuri* group for dichotomous analyses. There were 62 cases from control groups and 75 cases from *Radix Bupleuri* group for continuous analyses (**Figure 1**). There were no significantly changes for the outcomes of early study between *Radix Bupleuri* and the control group ($P > 0.05$). There was significantly greater change in the response at 3 months with *Radix Bupleuri* than that with the control group (OR = 3.22; **Table 2**) ($P < 0.05$).

The changes in outcomes diagnosed in the Radix Bupleuri group for continuous outcomes

For the depression analyses, heterogeneity was higher in the continuous outcomes than that in dichotomous outcomes while the pooled effect sizes are very similar and the effect sizes are smaller when the continuous outcomes are used [37]. **Table 3** showed compared outcomes in each trial. The changes were significantly greater in the *Radix Bupleuri* group than those in controls for MADRS, HADS-A, GAF scores and health-related quality of life survey RAND-36 score ($P < 0.05$) (**Table 3**). The patients who received *Radix Bupleuri* had greater improvement in depression symptoms (mean difference 4.72, 95% CI 0.69 to 9.12), anxiety symptoms (1.75, 95% CI 0.17 to 3.68) and general functioning (-4.95, 95% CI -9.18 to 1.37) at three-month follow-up via controls ($P < 0.05$) (**Table 3**). However, there were no significant changes for TAS-20 ($P > 0.05$).

More drop-out rates in control group that those in *Radix Bupleuri* group because of no motivation and other reasons (18 dropped out from the control group and 5 dropped out from *Radix Bupleuri* group). All the results suggested that *Radix Bupleuri* can be an effective way for improving depression in hemodialysis patients.

Radix Bupleuri improves the depression

The items of KICA-dep showed that *Radix Bupleuri* improves the depression in hemodialysis patients via those from control group (**Table 4**). This clinical examination was performed within one week according to ICD-10 and DSM-IV-TR criteria. Among the 11 items of

Table 3. Change of outcomes in the *Radix Bupleuri* group

Outcome	3-month follow-up, (n = 137)[a]				
	Mean (s. d.)	Change from baseline (s. d.)	Mean difference (95% CI)	T-test	P
Montgomery-Asberg Depression Rating Scale					
Control group	16.73 (9.46)	-2.24	4.72 (0.69 to 9.12)	2.31	0.02
Radix Bupleuri group	13.32 (8.25)	-11.36			
Hospital Anxiety and Depression Scale-Anxiety					
Control group	8.07 (4.26)	1.08	1.75 (0.17 to 3.68)	2.22	0.03
Radix Bupleuri group	6.59 (4.11)	-3.95			
Global Assessment of Functioning					
Control group	63.56 (9.74)	1.02	-4.95 (-9.18 to 1.37)	-2.17	0.03
Radix Bupleuri group	69.48 (9.16)	-11.42			
Toronto Alexithymia Scale-20					
Control group	48.53 (12.31)	-4.32	2.17 (-2.58 to 6.59)	0.96	0.46
Radix Bupleuri group	46.24 (11.99)	-5.87			
RAND-36					
Control group	61.34 (17.54)	-1.12	-4.61 (-11.32 to 2.75)	-2.39	0.04
Radix Bupleuri group	66.42 (19.83)	14.21			

[a]Control group n = 62; *Radix Bupleuri* group n = 75.

KICA-dep, the normal therapy with medicine could not improve the most depression of the patients there were no significant statistic differences for most cases (P > 0.05) except of two items (**Table 4**). Comparatively, *Radix Bupleuri* could improve the most depression of the patients there were significant statistic differences for most cases (P < 0.05) except of two items (**Table 4**). After the *Radix Bupleuri*, the number of slight depression increased while the number of heavy depression decreased (P < 0.05) (**Table 4**).

Protein levels of NGF and BDNF

The consists in 160 subjects before three-month follow-up and 137 cases after three-month follow-up. The socio-demographic was list in **Table 1**. The sample was balanced by age, gender, educational level, life habits and medication therapy. The results showed that Serum NGF levels were significantly higher in subjects accepted *Radix Bupleuri*s (178.64 ± 52.18 pg/mL) when compared to control (103.54 ± 31.23 pg/ml) (P < 0.01) (**Figure 2**). Similarly, serum BDNF levels were significantly higher in subjects accepted *Radix Bupleuri*s (1635.26 ± 121.66 pg/ml) when compared to control (516.38 ± 44.89 pg/ml) (P < 0.01) (**Figure 2**). All these results implied that *Radix Bupleuri* may promote the release of NGF and BDNF.

Association between a depression episode and the protein levels of NGF and BDNF

As showed in **Figure 3A** and **3B**, increasing levels of NGF and BDNF led to a decrease of the scores of MADRS and an increase of RAND-36 (**Figure 3C** and **3D**) (P < 0.01). There was a strong relationship between depression degrees and the levels of NGF or BDNF since all rho values were more than 0.5 or less than -0.5 according to the test of Spearman's rank correlation coefficient.

Discussion

The aim of this study is to investigate the effectiveness of *Radix Bupleuri* in the treatment of depression in hemodialysis patients and related molecular mechanism. Firstly, *Radix Bupleuri* is an effective way for the therapy of depression. Importantly, Radix Bupleuri therapy showed better results when compared to controls. The results also suggested that the *Radix Bupleuri* therapy makes full use of its advantages of anti-oxidant [38] activities, which promote the expression of BDNF and NGF [39, 40]. Secondly, it is difficult to detect the *Radix Bupleuri* contribution to the therapy for improving depression. Here computational methods with particular motivation to detect therapeutically-relevant phenomena, interpreted the possible outcome.

Table 4. The comparison for Prevalence of KICA-dep items among hemodialysis patients with DSM-IV-TR diagnosis of depression before and after three-month therapy

KICA-Dep Items:	Response	Control group			*Radix Bupleuri* group			Difference between two groups (*P* value)	
		Baseline N (%)	3 months N (%)	*P* value	Baseline N (%)	3 months N (%)	*P* value	Baseline	3 months
Felt down, sad, no good	Never	8 (10)	6 (9.7)	0.587	9 (11.3)	17 (22.5)	0.084	0.798	0.141
	Sometimes	48 (60)	36 (58.1)	0.582	46 (57.5)	45 (60.0)	0.183	0.748	0.588
	A lot	4 (5)	4 (6.5)	0.210	4 (5)	9 (12.5)	0.116	1	0.270
	All the same	20 (25)	16 (25.8)	0.899	21 (26.2)	4 (5.0)	**0.001**	0.856	**0.038**
Felt like doing things that you usually like doing	Never	10 (12.5)	7 (15.0)	0.826	9 (11.3)	19 (25.0)	**0.006**	0.807	**0.037**
	Sometimes	50 (62.5)	32 (67.5)	0.193	49 (61.2)	51 (68.0)	0.380	0.871	0.051
	A lot	13 (16.3)	6 (12.5)	0.499	14 (17.5)	3 (3.8)	**0.005**	0.833	**0.043**
	All the same	7 (8.7)	2 (5.0)	0.180	8 (10)	2 (2.5)	0.063	0.786	0.847
Had trouble going to sleep, staying asleep, or sleeping too much	Never	8 (10)	5 (8.1)	**0.044**	9 (11.3)	16 (21.3)	0.086	0.798	0.117
	Sometimes	48 (60)	28 (45.2)	0.185	49 (61.2)	47(62.5)	0.871	0.871	0316
	A lot	4 (5)	2 (3.8)	0.699	4 (5)	8(11.3)	0.148	1	0.072
	All the same	20 (25)	14 (22.6)	0.053	18 (22.5)	4 (5.0)	**0.001**	0.710	0.331
Felt tired or slack, and had no energy	Never	12 (15)	10 (20.0)	0.854	13 (16.3)	21 (27.5)	0.085	0.828	0.098
	Sometimes	40 (50)	28 (57.5)	0.341	39 (48.7)	37 (49.3)	0.874	0.874	0.341
	A lot	20 (25)	8 (17.5)	0.246	19 (23.8)	14 (18.8)	0.440	0.854	0.837
	All the same	8 (10)	2 (5.0)	0.230	9 (11.2)	3 (3.8)	0.072	0.798	0.699
Eating too much or eating only a little bit.	Never	47 (58.7)	34 (71.3)	0.097	47 (58.8)	63 (83.8)	**0.001**	1	**0.001**
	Sometimes	13 (16.2)	8 (17.5)	0.833	11 (13.7)	9 (12.5)	0.815	0.658	0.376
	A lot	16 (20)	3 (6.3)	**0.010**	17 (21.3)	2 (2.5)	**0.001**	0.845	0.246
	All the same	14 (17.5)	2 (5.0)	**0.012**	15 (18.7)	1 (1.3)	**0.001**	0.837	0.173
Felt bad or shamed that you let yourself or your family down.	Never	28 (35)	24 (38.7)	0.514	30 (37.5)	45 (60.0)	**0.004**	0.742	**0.013**
	Sometimes	25 (31.2)	17 (35.0)	0.614	24 (30)	19 (25.0)	0.479	0.864	0.168
	A lot	17 (21.2)	9 (18.8)	0.693	18 (22.5)	9 (12.5)	0.096	0.848	0.276
	All the same	10 (12.5)	3 (6.3)	0.175	12 (15)	2 (2.5)	**0.005**	0.646	0.246
Had trouble paying attention or concentrating on things	Never	16 (20)	12 (25.0)	0.449	15 (18.8)	28 (37.5)	**0.008**	0.841	**0.021**
	Sometimes	41 (51.3)	34 (54.8)	0.526	39 (48.7)	33 (43.8)	0.526	0.752	0.207
	A lot	14 (17.5)	7 (13.8)	0.514	15 (18.7)	11 (15.0)	0.391	0.837	0.822
	All the same	9 (11.2)	2 (5.0)	0.148	11 (13.8)	3 (3.8)	0.025	0.633	0.699

Been told that you are speaking or moving too slowly or fast	Never	45 (56.3)	42 (67.7)	0.194	44 (55)	63 (83.8)	**0.001**	0.874	**0.025**
	Sometimes	16 (20)	10 (21.3)	0.845	17 (21.2)	10 (13.8)	0.212	0.845	0.212
	A lot	13 (16.2)	4 (8.8)	0.151	13 (16.2)	1 (1.3)	**0.001**	1	**0.030**
	All the same	6 (7.5)	2 (3.8)	0.303	6 (7.5)	1(1.3)	0.053	1	0.311
Had thoughts that you would be better off dead	Never	23 (28.8)	16 (32.5)	0.604	21 (26.3)	35 (46.3)	**0.008**	0.723	**0.012**
	Sometimes	37 (46.2)	23 (48.8)	0.752	37 (46.2)	29 (38.8)	0.337	1	0.202
	A lot	12 (15)	7 (13.8)	0.822	13 (16.2)	10 (13.8)	0.658	0.828	1
	All the same	8 (10)	2 (5.0)	0.230	9 (11.2)	1 (1.3)	**0.009**	0.798	0.173
Thought of hurting yourself	Never	25 (31.3)	10 (15.5)	**0.001**	24 (30)	56 (74.7)	**0.001**	0.864	**0.001**
	Sometimes	26 (32.5)	17 (35.0)	0.738	25 (31.2)	11 (15.0)	**0.015**	0.865	**0.003**
	A lot	22 (27.5)	10 (21.3)	0.357	13(16.2)	8 (11.3)	0.358	0.085	0.086
	All the same	7 (8.7)	11 (17.7)	**0.001**	8 (10)	0(0.0)	**0.004**	0.786	**0.023**
Felt angry	Never	12 (15)	4 (6.5)	**0.001**	11 (13.8)	38 (51.3)	**0.001**	0.822	**0.001**
	Sometimes	44 (55)	20 (32.2)	0.522	43 (53.7)	34 (45.0)	0.268	0.874	0.057
	A lot	14 (17.5)	6 (12.5)	0.376	16 (20)	2 (2.5)	**0.001**	0.685	**0.016**
	All the same	10 (12.5)	18 (19.0)	**0.001**	10 (12.5)	1 (1.3)	**0.005**	1	**0.030**

Note: bold number stands for $P < 0.05$.

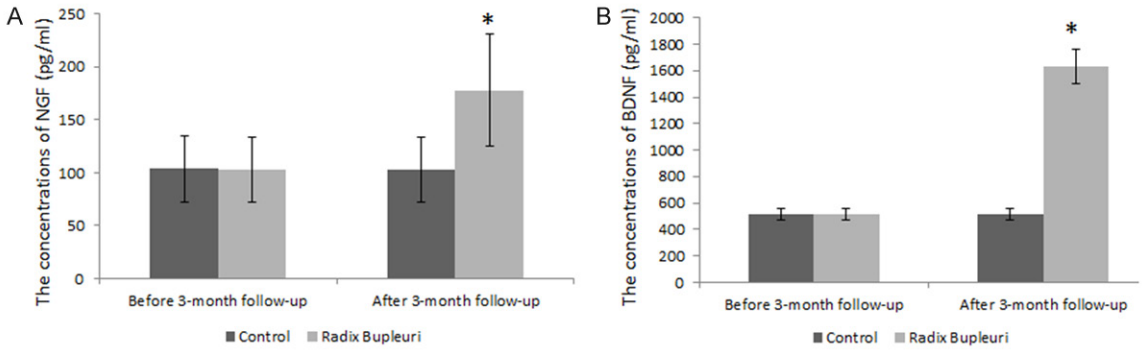

Figure 2. Serum NGF and BDNF levels in control subjects and *Radix Bupleuri* group before and after three-month follow-up. A. Serum NGF levels (pg/mL) in control subjects and *Radix Bupleuri* group before and after three-month follow-up. B. Serum BDNF levels (pg/mL) in control subjects and *Radix Bupleuri* group before and after three-month follow-up. Comparative analyses were carried out using ANOVA followed by Duncan test. Values are expressed as mean ± SD. N = 65 cases in control group and 72 cases in *Radix Bupleuri* group. **P* < 0.01 via controls.

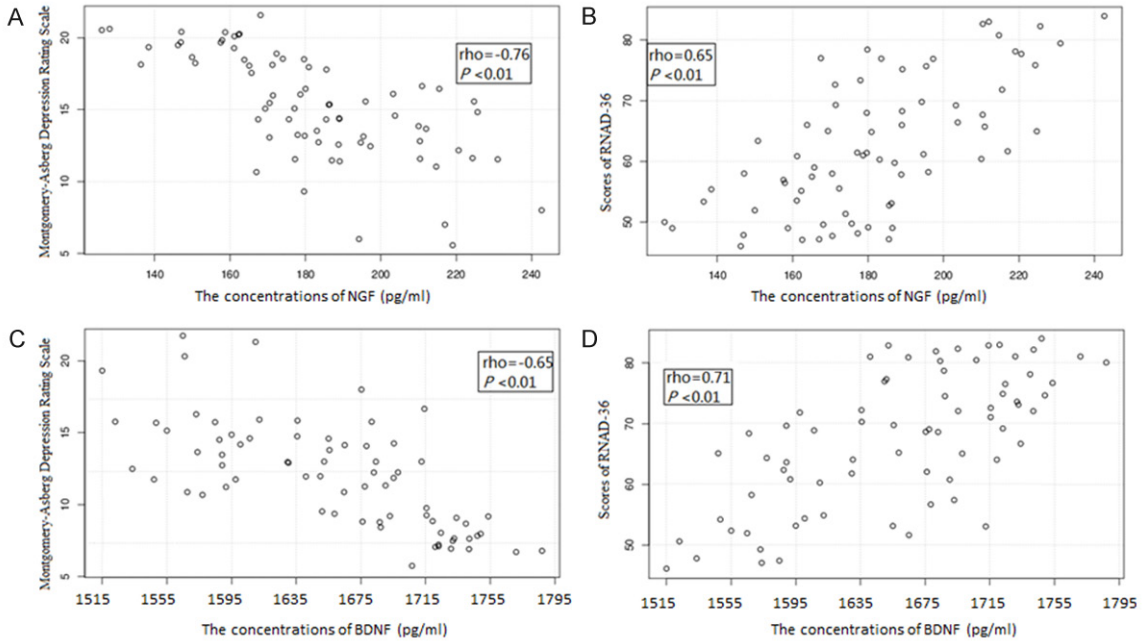

Figure 3. The correlation between the degrees of depression (Montgomery-Asberg Depression Rating Scale or scores of RAND-36) and the levels of NGF or BDNF in *Radix Bupleuri* group. A. The correlation between Montgomery-Asberg Depression Rating Scale and the levels of NGF. B. The correlation between scores of RAND-36 and the levels of NGF. C. The correlation between Montgomery-Asberg Depression Rating Scale and the levels of BDNF. D. The correlation between scores of RAND-36 and the levels of BDNF. Statistical analysis was performed using Spearman's rank correlation test. There was a strong negative relationship if rho value fell between -1 and -0.5. In contrast, there was a strong positive relationship if rho value fell between 0.5 and 1. There is significant difference if *P* value is less than 0.01. N = 72 cases.

The strengths of the paper were its methodology of the clinical technique. Furthermore, *Radix Bupleuri* therapy was established for the different characters of individuals. On the other hand, the combination of various techniques was utilized. Here, brain imaging and self-ratings, computational analysis, and psychiatric assessment was undertaken, resulting in a complementary and myriad measures access to various underlying determinants of a treatment. Some brain imaging techniques, such as fMRI and PET, could provide distinct spatial

information, but which could not provide time and event-related correlations. Depression has been reported to be correlated with a hypo-activation of left brain activity and a hypo-inactivation of right brain activity [41], which can be ameliorated with the time going after treatment.

Alexithymia, defecting in interpreting and regulating feelings [42], typically occurs with depression [43] and was considered here. However, Alexithymia is a complex brain disorder and considered a cluster of deficits in the recognition [44]. Here we found that, *Radix Bupleuri* was not an effective way for the treatment of alexithymia compared with that from the control group ($P > 0.05$) (**Table 3**). In any way, *Radix Bupleuri* can enhance the health-related quality of life ($P < 0.05$) (**Table 3**).

Radix Bupleuri therapy turns out to be effective it offers the best way for the therapy in the patients with depression. *Radix Bupleuri* therapy offers an appropriate option and will be considered in future. In addition, evoking and dealing with emotions is usually related with *Radix Bupleuri*, which fit the treatment of emotional disorders just like depression. In order to gain an insight into the effectiveness of *Radix Bupleuri*, we also aim to develop novel analytical methods for clinical purposes. Meanwhile, the study will provide valuable information for the therapy of widespread depression.

Radix Bupleuri often affects the progression of depression but the molecular mechanism remains unknown. Reduced levels of NGF have been reported to increase the risk of depression [16, 17] while BDNF has anti-depression functions [18, 19]. However, the clinical relevance of *Radix Bupleuri* and the levels of NGF and BDNF are still unknown. Here, we firstly reported that *Radix Bupleuri* ameliorate the depression by affecting the serum levels of NGF and BDNF. We found the relationships between *Radix Bupleuri*s and levels of NGF and BDNF. This was the first report showing that lower concentration of NGF and BDNF may be specific for the depressive state in depression patients. NGF and BDNF are potential negative biomarkers for adjuvant diagnosis and therapy of a depressive episode. However further studies are necessary to define a functional role of NGF and BDNF in the development of depression.

Except that the patients receive Radix Bupleuri treatment for depression, the following clinical cares should be paid to reduce the patient's un-favored emotions and to mitigate the occurrence of depression: 1) it is important to maintain and strengthen their mental health via encouragement and other ways to understand the patients' work, family, economic conditions, concerns and requirements. With the information, specific targeted psychological counseling can be performed according to individual's situation. With emotional and social support, the severity of depression of these patients can be reduced. 2) It is necessary to improve the dialysis efficiency and increase the frequency of dialysis. These methods can be used to remove the toxin molecules and reduce complications, such as cardiovascular disease, anemia, malnutrition, renal bone disease, neurological diseases and other risk factors which affect the survival of patients. 3) It is critical to strengthen nurses' accountability. For example, close observation and timely treatment for acute complications should be performed, such as the imbalance syndrome, muscle spasms, hypotension, and arrhythmia. Clotting and air embolism should be avoided in bleeding dialysis. These methods can help reduce patients' uncomfortable feelings. Meanwhile, all patients should be guided to control diet to avoid weight gain, which will lead to heart failure, hyperkalemia and so on. It is also critical to improve patients' mental situation and complications following the treatment of dialysis.

Certainly, the present trial still has its limitations in a statistical sense. The sample size was sufficient to detect an effect in the primary outcome at three months, but not at longer period although we found the effect tended to persist. Second, the present trial was the *Radix Bupleuri*. We still don't know whether the *Radix Bupleuri* group is better than other medicine therapy. At least, we still believe that *Radix Bupleuri* may be more suitable for a few populations only received medicine therapy. Finally, the *Radix Bupleuri* is still hard to be performed in most areas in China. How to develop and standard way or spread *Radix Bupleuri* for the large population in China is still needed to be explored in future. In any way, more research is needed to explore the feasible domain specific benefits of combination therapy as compared to other therapies. Taking the consideration,

certain definitions and methodological solutions can be achieved.

Conclusions

The results of the work suggested that *Radix Bupleuri* can be effective methods as nursing intervention contributing to the improvement of quality of life in hemodialysis patients by reducing their depression. *Radix Bupleuri* ameliorates the symptoms of depression by increasing the level of NGF and BDNF. Thus, *Radix Bupleuri* should be developed as a new drug for the treatment of depression disorder.

Disclosure of conflict of interest

None.

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